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Gold standard validation of fecal egg counts and larval cultures as diagnostic tools in horses

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Introduction

- Traditional parasitological methods for counting nematode eggs in feces and performing larval cultures to count and identify third stage strongyle larvae are widely used in equine establishments.
- The increasing levels of anthelmintic resistance reported world-wide have lead to a change in recommended paradigm with more emphasis on parasite surveillance and diagnosis.
- Despite the widespread usage and importance of egg counts and larval cultures no published report has ever attempted to validate these techniques for diagnostic usage. Such studies would yield crucial information to help interpreting the results of these tests.
- The purpose with the present study was to utilize the database generated at University of Kentucky to retrospectively validate fecal egg counts and larval culture counts against total luminal worm counts performed on necropsies of horses over a period of five decades.

Materials and Methods

- The data comprised total luminal worm counts, fecal egg counts (FEC) and larval culture results from 693 horses used for critical and controlled tests between 1960 and 2005 (Lyons et al., 1983).
- All procedures were carried out by the same individuals in the Kentucky research group and the same protocols were followed throughout the period.
- Mean and median age of horses was 1.5 and 1 year, respectively.
- 10% were geldings, 38% mares, and 52% stallions.
- A modified Stoll technique with a detection limit of 10 eggs per gram (EPG) was used for generating strongyle and ascarid egg counts.
- Larval cultures were performed individually from 100 g of feces mixed with sphagnum moss and incubated for one week in an incubator set at 27 ℃ and 80 % relative humidity (Drudge et al, 1963).
- Third stage larvae were identified according to Russell (1948).
- Sensitivity, specificity, positive and negative predictive values, and receiver operator characteristic (ROC) curves were generated for the larval cultures and ascarid egg counts.
- For strongyle egg counts, different cutoff values of strongyle egg counts for predicting adult worm burdens. This was done with Box-Whisker plots and comparisons between the two groups (i.e., above/below cutoff value) using a Mann-Whitney U test with ties and Wilcoxon-sign rank test.

Results

- All tested cutoff values in the range on 100-500 EPG yielded significantly lower strongyle worm burdens in horses below the egg count cutoff value. For cutoffs above 500 EPG, no differences in worm counts were found (figure 1).
- ROC curves for the larval culture and ascarid egg count are presented in figures 2-4.
- Table 1 presents specificities, sensitivities, predictive values and likelihood ratios for the same three diagnostic tests.
- No linear correlations were found between larval/egg counts and worm burdens

Discussion

- Horses with strongyle egg counts in or below the range of 100-500 EPG had significantly lower worm burdens than the remainder of horses. This leads support for usage of selective therapy.
- Mediocre sensitivities, but high specificities suggest that a negative result should be interpreted with caution. For any test in table 2, the false negative rate was about 25-30%.
- Larval cultures performed better for diagnosing *S. edentatus* than *S. vulgaris*, but no obvious explanation could be found.
- The lack of correlation between egg/larval counts and worm burdens indicates that the tests have more value as qualitative diagnostic tools, while the actual count yields little information about the size of the worm burden.
- All tests are useful for diagnosing luminal worm burdens, but negative tests may be repeated to increase diagnostic sensitivity.

References

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Lyons, E.T., Drudge, J.H., Tolliver, S.C., 1983. Controlled tests with fenbendazole in equids: Special interest on activity of multiple doses against natural infections of migrating stages of strongyles. Am. J. Vet. Res. 44, 1058-1063.
Russell, A.F., 1948. The development of helminthiasis in Thoroughbred foals. J. Comp. Path. Therap. 58, 107-127.

Table 1. Diagnostic performance of larval cultures and the *Paracaris* egg count. 95% confidence intervals are given in parantheses for each parameter.

	Larval culture for detecting *Strongylus vulgaris*	Larval culture for detecting *Strongylus edentatus*	*Parascaris* egg count for detecting *Parascaris equorom*
Sensitivity	0.73 (0.69-0.76)	0.75 (0.71-0.79)	0.72 (0.68-0.76)
Specificity	0.84 (0.76-0.90)	0.96 (0.92-0.98)	0.94 (0.90-0.96)
Positive Predictive Value	0.96 (0.94-0.98)	0.98 (0.96-0.99)	0.95 (0.93-0.97)
Negative Predictive Value	0.37 (0.34-0.40)	0.60 (0.57-0.61)	0.66 (0.64-0.68)
Likelihood ratio – positive	4.59 (2.92-7.21)	20.33 (9.25-44.67)	12.11 (7.26-20.20)
Likelihood ratio – negative	0.33 (0.28-0.38)	0.26 (0.22-0.30)	0.30 (0.25-0.35)

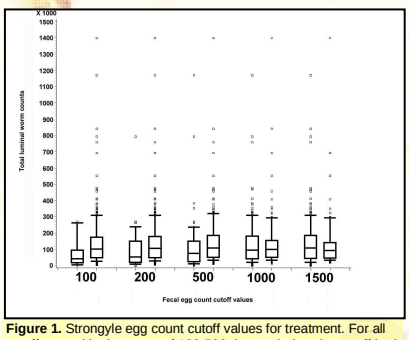

Figure 1. Strongyle egg count cutoff values for treatment. For all cutoffs tested in the range of 100-500, horses below the cutoff had significantly smaller worm burdens than the remainder of horses.

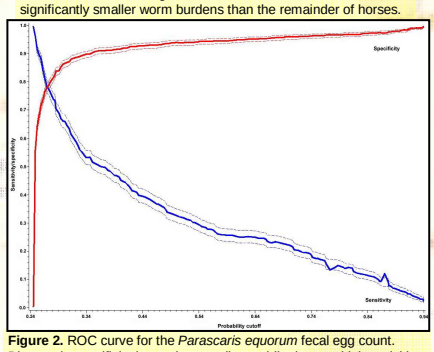

Figure 2. ROC curve for the *Parascaris equorum* fecal egg count. Diagnostic specificity is good to excellent, while the sensitivity quickly declines to mediocre. Detection of one egg corresponds to 0.28.

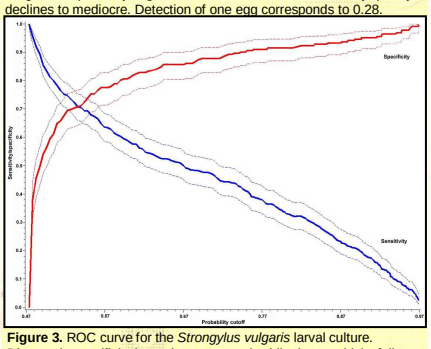

Figure 3. ROC curve for the *Strongylus vulgaris* larval culture. Diagnostic specificity is moderate to good, while the sensitivity falls below 0.80 before the specificity reaches 0.90.

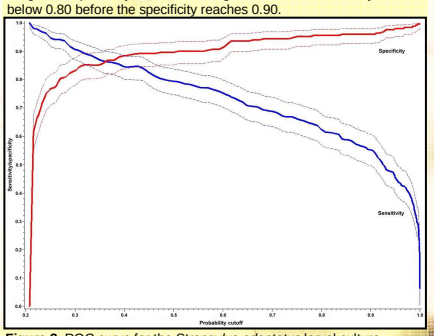

Figure 3. ROC curve for the *Strongylus edentatus* larval culture. Diagnostic specificity is good to excellent, while the sensitivity remains above 0.85 at the lower probability levels before it gadually declines.